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Corynebacterial Necrohemorrhagic Cystitis in Two Female Macaques

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We describe severe necrohemorrhagic cystitis in a female rhesus macaque and a female cynomolgus macaque due to colonization of the urinary bladder by *Corynebacterium sp*. Clinically, both macaques presented with perineal bleeding and depression and, despite extensive and prolonged treatment, succumbed to the disease. At necropsy, the contents of the urinary bladders in both cases were hemorrhagic to greenish black, and the bladder mucosa was necrotic. The major microscopic finding in each case was transmural necrohemorrhagic cystitis, with vasculitis, fibrin thrombi, and myriad gram-positive coryneform bacilli. *Corynebacterium renale*, *Streptococcus acidominimus*, and *S. oralis* were cultured from the urinary bladder of the rhesus macaque, and a nondiphtheritic *Corynebacterium* was cultured from the urinary bladder of the cynomolgus macaque. Neither animal had any other noteworthy pathologic lesions unrelated to bacterial cystitis. Corynebacterial necrohemorrhagic cystitis therefore was determined to be the cause of death in both animals. To our knowledge, this is the first report of corynebacterial cystitis in nonhuman primates.

Abbreviations: USAMRIID, United States Army Medical Research Institute of Infectious Diseases

Corynebacterial infections are important causes of disease, morbidity, and death in humans and various wild and domestic animal species. *Corynebacterium diphtheriae* causes diphtheria in humans, a highly contagious upper respiratory tract disease characterized by a pseudomembrane within the tonsil, pharynx, and nose. However, many nondiphtheritic coryneform bacteria are a component of the bacterial flora in the skin and mucous membranes, ubiquitous environmental organisms, and potential opportunistic pathogens.[3-5] With few exceptions, the opportunistic infections caused by nondiphtheritic coryneform bacteria are characterized by necrotizing tissue lesions with suppurative inflammation in the affected host.[4,7]

Of the nondiphtheritic coryneform bacteria, several members of the *Corynebacterium renale* group, including *C. renale*, *C. pilosum*, and *C. cystitidis*, are opportunistic urinary tract pathogens in domestic animals and natural causes of cystitis, ureteritis, and ascending pyelonephritis in cattle. This condition in the bovid is known as bacillary pyelonephritis.[3,5,7] Animals may become predisposed to bacillary pyelonephritis through physical or chemical damage to the lower genitourinary tract caused by dystocia, urinary bladder paralysis, and urinary catheterization. Urinary tract infections are more common in female animals. These predisposing factors disrupt the host's natural defenses, such as the mucosal barrier, and may allow initial colonization of tissue with coryneform bacteria. Hemorrhagic urethritis, cystitis, and pyelonephritis develop as a result of ascending urinary tract infection.[3,5,8,10] In this report we describe 2 cases of necrohemorrhagic cystitis from macaques in our nonhuman primate colony that had clinicopathologic findings similar to those found in cattle diagnosed with bacillary pyelonephritis; *Corynebacterium* spp. were cultured from both animals, and *C. renale* was identified in the isolate from the rhesus macaque.

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Case Reports

Both macaques described were members of the colony at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID; Fort Detrick, MD) and were awaiting placement on a protocol. Research at USAMRIID is conducted in compliance with the Animal Welfare Act and other principles stated in the *Guide for the Care and Use of Laboratory Animals*.[6] USAMRIID is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Case 1. An adult female rhesus macaque (*Macaca mulatta*) presented clinically with a distended abdomen and perineal bleeding. During physical examinations by the laboratory animal veterinarian, the animal was hypothermic (30.6 °C), with abdominal distention and a blood-stained perineum. Abdominocentesis was performed and the sampled fluid was suspicious for bloody urine. A clinical diagnosis of urethral blockage was made, and an emergency exploratory laparotomy was performed. At surgery, the urinary bladder was severely distended and dark red to black. Cystotomy was performed, and the urinary bladder was evacuated and lavaged with sterile saline. A gritty and mucoid plug was removed from the cranial urethra, and the animal recovered from surgery without incident. Several hours after recovery, the animal's condition rapidly declined, and she died that evening.

Case 2. Animal caretakers reported that a 4-y-old, female, cynomolgus macaque (*Macaca fascicularis*) was in estrous and not eating well. During physical examination by the laboratory animal veterinarian, the animal was lethargic and dehydrated and the perineum contained a moderate amount of bloody discharge. Initial treatment included aspirin and oral electrolyte replacement. There was no clinical improvement, and the regimen was changed to intramuscular flunixin meglumine, intramuscular enrofloxacin, and 300 ml of subcutaneous fluids. The animal's physical condition continued to deteriorate, and she became unresponsive and hypothermic. In addition, increased capillary refill time, bilateral nystagmus, mucoid diarrhea, persistent blood-stained perineum (presumed estrous), and considerable abdominal pain were observed. The macaque

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14. ABSTRACT

We describe severe necrohemorrhagic cystitis in a female rhesus macaque and a female cynomolgus macaque due to colonization of the urinary bladder by Corynebacterium sp. Clinically, both macaques presented with perineal bleeding and depression and, despite extensive and prolonged treatment, succumbed to the disease. At necropsy, the contents of the urinary bladders in both cases were hemorrhagic to greenish black, and the bladder mucosa was necrotic. The major microscopic fi nding in each case was transmural necrohemorrhagic cystitis, with vasculitis, fi brin thrombi, and myriad gram-positive coryneform bacilli. Corynebacterium renale, Streptococcus acidominimus, and S. oralis were cultured from the urinary bladder of the rhesus macaque, and a nondiphtheritic Corynebacterium was cultured from the urinary bladder of the cynomolgus macaque. Neither animal had any other noteworthy pathologic lesions unrelated to bacterial cystitis. Corynebacterial necrohemorrhagic cystitis therefore was determined to be the cause of death in both animals. To our knowledge, this is the first report of corynebacterial cystitis in nonhuman primates.

15. SUBJECT TERMS

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was treated for shock and suspected sepsis by using intravenous fluids with 2.75% dextrose and continued intramuscular enrofloxacin and flunixin meglumine. Despite these aggressive therapies, the animal died subsequently.

Results

Complete necropsies were performed on each animal in a Biosafety Level 2 necropsy facility. A complete set of tissues were immersion-fixed in 10% neutral buffered formalin for at least 2 d and were processed for histopathology. Sections prepared for examination by light microscopy were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Select tissues were stained for bacteria by the Gram–Twort method. Briefly, deparaffinized sections were immersed in crystal violet for 1 min and stained with Lugol's iodine and neutral red–fast green.

Case 1 (rhesus macaque; Figure 1). At necropsy, a bloody discharge from the urethra was noted, and the hair surrounding the perineum and ventral aspect of the tail was blood-stained. The wall of the urinary bladder was diffusely red-black (that is, the bladder wall was hemorrhagic), and the lumen contained approximately 15 ml of thick, partially coagulated, viscous, bloody fluid. The mucosa was friable and sloughed easily when manipulated (that is, the mucosa was necrotic). The abdominal mesentery was focally adhered to the apex of the urinary bladder, and both ureters were diffusely enlarged to 3 times normal size and measured approximately 5 mm in diameter (that is, bilateral hydroureter was present). The abdominal cavity contained approximately 50 ml of red-brown fluid (serosanguineous ascites); the thoracic cavity had approximately 25 ml of clear, amber fluid (serous pleural effusion); and the pericardial sac contained approximately 10 ml of red fluid (serosanguineous pericardial effusion). The heart blood was extremely thin and watery and failed to clot. Aerobic microbial culture of the urinary bladder contents yielded mixed growth of *C. renale* and *Streptococcus acidominimus*. After broth enrichment, *S. oralis* was cultured from the fluid in the pericardial sac and from the hemorrhagic contents in the urinary bladder.

Case 2 (cynomolgus macaque, Figure 2). Gross necropsy findings included moderate bloody discharge from the vulva. The mucus membranes of the lips, mouth, conjunctiva, and sclera were diffusely pale white (that is, anemic). The urinary bladder was diffusely green to black, distended, and contained a mixture of dark red to greenish black fluid that was irregularly granular to clumped (that is, the bladder contained clotted blood and bloody urine). The mucosa and muscular wall of the urinary bladder were friable and easily torn (that is, the tissue was necrotic). The hemorrhagic contents from the urinary bladder and the heart blood were sampled at necropsy for microbial culture; the heart blood was extremely thin and watery during collection. Aerobic bacterial culture of the contents of the urinary bladder demonstrated growth of a nondiphtheritic *Corynebacterium* that was not further speciated by the laboratory; aerobic microbial culture of the heart blood yielded no growth after 72 h of incubation at 37 °C.

The histopathologic features of the urinary bladder lesions were similar in both animals. The most prominent histologic lesion was transmural necrohemorrhagic to fibrinosuppurative cystitis that was diffuse and severe. The urinary bladder mucosa was replaced by a fibrinonecrotic (diphtheritic) membrane or pseudomembrane, composed of abundant fibrin, hemorrhage, necrotic transitional epithelial cells, degenerate neutrophils, and many gram-positive bacilli. The wall of the urinary bladder (submucosa, muscular tunic, and serosa) was expanded to 3 to 4 times the normal thickness by abundant fibrin, edema, hemorrhage, and viable and degenerate neutrophils. Blood vessels were congested, and there was multifocal necrotizing vasculitis with many fibrin thrombi. Tissue Gram staining identified many coryneform, gram-positive, bacillary organisms within the pseudomembrane, mucosa, and submucosa, consistent with the culture results of *Corynebacterium* species in both cases. The histologic lesions in the urinary bladder of these animals were attributed primarily to corynebacterial infection. In case 1, gram-positive cocci also were revealed after Gram staining of the necrotic bladder mucosa, consistent with the culture of *S. acidominimus*.

Additional noteworthy histologic lesions in the rhesus macaque included acute multifocal necrotizing myocarditis in the left and right ventricles. Corynebacterial hemorrhagic cystitis and subsequent endotoxemia was considered to be the primary cause of death in this case. Additional histologic findings in the cynomolgus macaque included acute to subacute fibrinous polyserositis of the urinary bladder, stomach, pancreas and mesentery; the inflammatory lesions on the serosal surfaces of the viscera were attributed to leakage of contents from the necrotic urinary bladder into the abdomen (that is, peritonitis was present). Severe anemia and the systemic cardiovascular effects (that is, shock) due to necrotizing corynebacterial cystitis were considered to be the cause of death in this case.

Discussion

To our knowledge, cystitis in nonhuman primates due to infection with *Corynebacterium spp*. has not been reported previously. Here, we describe *Corynebacterium*-induced necrohemorrhagic cystitis that affected 2 macaques in our nonhuman primate colony. These cases had similar signalment, clinical presentation, and pathologic findings. In particular, key features included adult female macaque with no prior medical history of illness; no known predisposing factors to urinary tract disease; protracted estrous bleeding; rapidly declining clinical condition eventually leading to shock and death; gross necropsy evidence of severe, diffuse cystitis; microscopic diagnoses of transmural necrohemorrhagic cystitis, with vasculitis, fibrin thrombi, and myriad gram-positive coryneform bacilli; and positive culture of *Corynebacterium spp.* These cases occurred within a year of each other, and no other predisposing factors were identified (signalment, housing, treatments, medical history, vendor source), and the source of infection remains unknown.

The findings in these cases have similarities to corynebacterial urinary tract infections in other animal species, including natural and experimental infections in cattle, goats, mice and rats.[1-3,9] Infection with *C. renale* group bacteria in cattle, in particular, can affect all or part of the urinary tract. In the urinary bladder, typical lesions include mural thickening from infiltrating leukocytes and hemorrhage, vasculitis and fibrin thrombi, mucosal necrosis, ulceration and perforation, hemorrhage, and replacement of the mucosa by a fibrinonecrotic (diphtheritic) membrane.[3,5] These features were characteristic of the 2 macaques from our facility.

Several bacterial virulence factors may predispose animals to infection and progression of necrohemorrhagic urinary system lesions due to *C. renale* group organisms. These organisms have pili, allowing attachment to the urogenital mucosa and facilitating ascension of microbes into the bladder and kidneys. The bacteria produce urease and hydrolyze urea, which may contribute to extensive ulceration of the mucosa and necrosis in the affected tissues.[2,5,7,10] Therefore, high-protein diets and subsequently elevated urinary urea levels may predispose animals

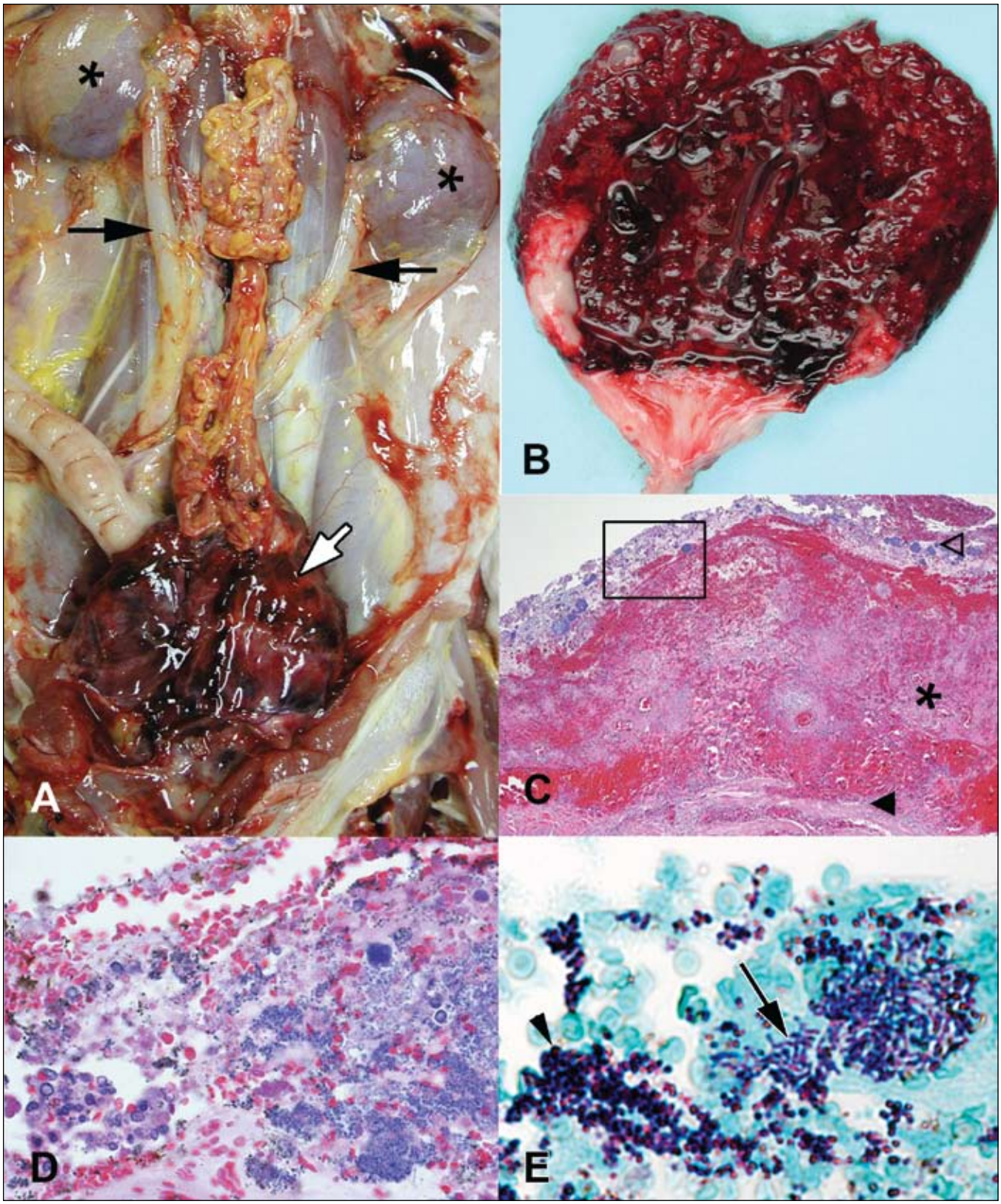

Figure 1. Case 1. (A) Kidneys (asterisks), ureters (black arrows), urinary bladder (white arrow), and colon. Note the bilaterally enlarged ureters consistent with hydroureter and the mottled red and black urinary bladder. (B) Urinary bladder. The mucosal surface is thickened, irregular, and varies in color from red to black (hemorrhage, edema, and necrosis). (C) Urinary bladder. The mucosa (open arrowhead), submucosa (asterisk), and muscular tunic (black arrowhead) of the urinary bladder are expanded markedly and disrupted by hemorrhage, edema, and inflammation. The mucosal surface is replaced by a pseudomembrane composed of fibrin, hemorrhage, cellular debris, and bacterial colonies. Hematoxylin and eosin stain; magnification, ×2. (D) Urinary bladder. Enlargement of boxed area of panel C, showing colonies of bacteria and erythrocytes. Hematoxylin and eosin stain; magnification, ×60. (E) Urinary bladder. Gram stain of mucosa illustrating both coryneform (arrow) and coccoid (arrowhead) gram-positive bacteria. Gram–Twort stain; magnification, ×100.

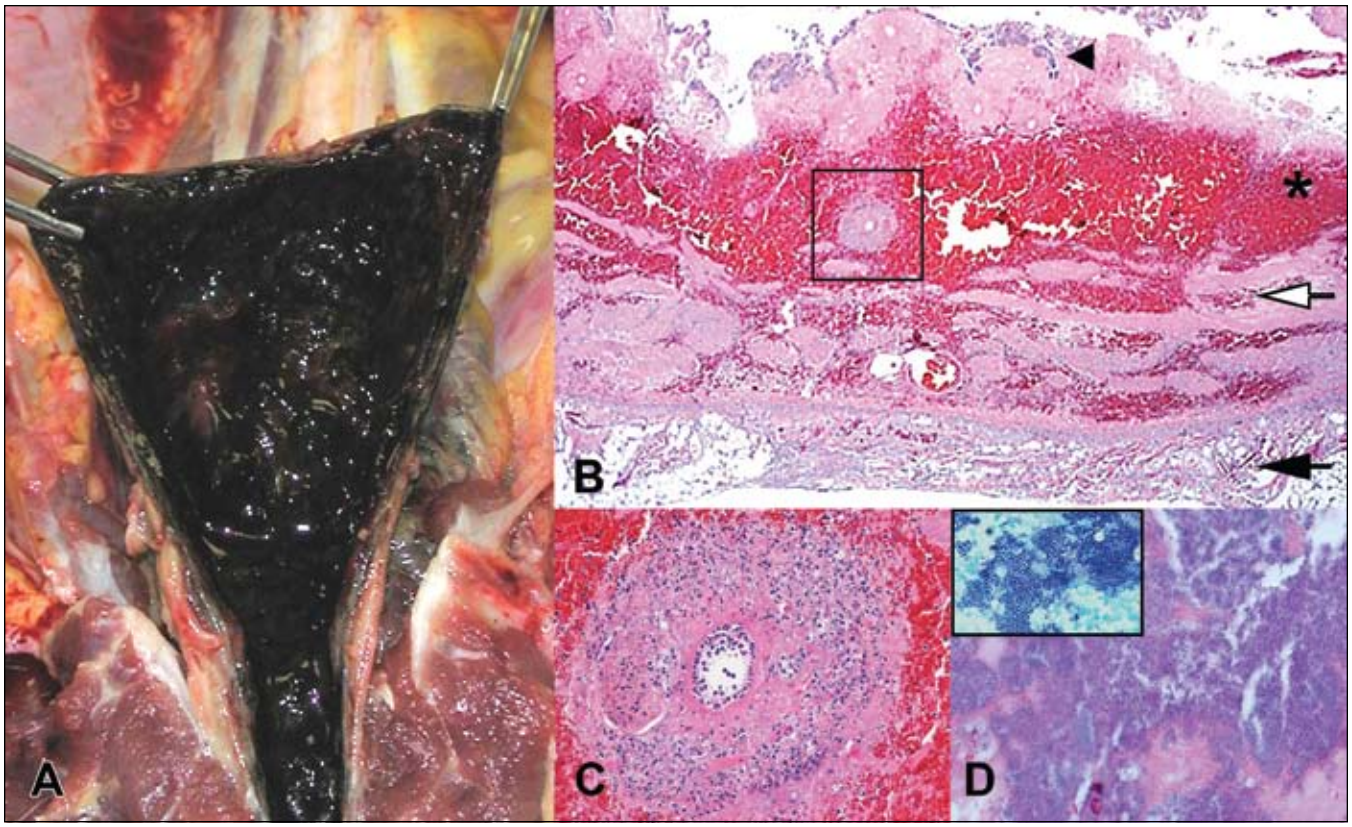

Figure 2. Case 2. (A) Urinary bladder. The mucosal surface is thickened, irregular, friable, and black, consistent with necrohemorrhagic cystitis. (B) Urinary bladder. All tunics, mucosa (black arrowhead), submucosa (black asterisk), muscular (white arrow), and serosa (black arrow) are expanded and disrupted by hemorrhage, edema, and inflammation. Note the isolation of individual muscle bundles (white arrow) as they are surrounded by hemorrhage and inflammation. The mucosal surface is covered by a pseudomembrane composed of fibrin, hemorrhage, cellular debris, and bacterial colonies. Hematoxylin and eosin stain; magnification, ×2. (C) Urinary bladder. Enlargement of submucosal boxed area in panel B, showing necrotizing vasculitis of a submucosal blood vessel. Hematoxylin and eosin stain; magnification, ×20. (D) Urinary bladder. Enlargement of mucosa showing colonies of gram-positive coryneform bacteria. Hematoxylin and eosin and Gram–Twort (box insert) stains; magnification, ×60.

to disease because of the bacteria's ability to hydrolyze urea.[7]

Various host factors can predispose animals to infection. For example, female cattle are more predisposed to infection than are male, due to anatomic structure, hormonal influences, and risks associated with pregnancy or iatrogenic procedures; infection in bulls is rare.[5] Females have short urethras, which decreases the anatomic barrier bacteria must overcome to reach the urinary bladder and kidneys, and urethral trauma associated with dystocia or catheterization may serve as initiating events for infection.[5,8,10] Hormone-induced changes in female animals may serve as predisposing factors; high estrogen levels may affect the functional integrity of the epithelium in the urethra and urinary bladder, and cattle with high estrogen levels that graze pastures are reportedly prone to infection with *C. renale*.[5,7] In some species, such as pigs, estrogen causes an elevation in the urine pH, which may produce an alkaline environment optimal for expression of bacterial pili and enhanced microbial survival and proliferation.[5,10] Spontaneous and experimentally induced *C. renale* infections in animals frequently are associated with alkaline urine, although this pattern may reflect postinfection bacterial hydrolysis of urea and production of ammonia rather than preexisting alkaluria.[2,5,10]

The histopathologic findings of necrotizing and hemorrhagic cystitis, and the microbial culture results from the contents of the urinary bladder in both animals support the underlying cause of death as complications from infection by nondiphtheritic coryneform bacteria. Gram-positive bacilli consistent with corynebacteria were present in the affected mucosa of the urinary bladder in both macaques.

In case 1 (rhesus macaque), the isolated coryneform organism was identified as *C. renale*; because of laboratory constraints, the organism cultured from the urine sample from case no. 2 (cynomolgus macaque) was identified only as a nondiphtheritic corynebacterium. However, based on the similarities of the clinicopathologic findings in both macaques and similarities to the descriptions of cattle infected with *C. renale*, we suspect both macaques were infected with members of the *C. renale* group. In cases of cystitis due to *C. renale* group bacteria in cattle, infections are usually mixed with other bacteria.[5] Similarly, microbial culture of the contents of the urinary bladder from the rhesus macaque yielded both *C. renale* and *S. acidominimus*, and both gram-positive cocci and coryneform bacilli were present histologically in the lesions in the urinary bladder.

In summary, the signalment, clinical history, pathologic findings, and microbial culture results in these 2 adult female macaques with necrohemorrhagic cystitis show similarities to spontaneous and experimentally induced corynebacterial urinary tract disease in several animal species. Severe urinary tract infection due to corynebacteria should be included in the clinical differential diagnosis for protracted vaginal bleeding in macaques.

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